Docket No.: ICON 3.3-001
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Kuchuk et al.

Application No.: 09/980,542 | Confirmation No.:

Filed: March 4, 2002 | Art Unit: 1638

For: PROCESS OF RAPID VARIETY-INDEPENDENT PLANT TRANSFORMATION | Examiner: Georgia L. Helmer

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. Victor Klimyuk, do declare as follows:

1. I hold a number of degrees including a Masters of Science from Kiev University, 1980, and a Ph.D. from the Institute of Cell Biology and Genetic Engineering, in Kiev, 1987. I am currently Director Development at Icon Genetics, a position I have occupied after acquisition of our company by Bayer AG at the beginning of January 2006. Before the acquisition, I was the Chief Scientific Officer of Icon Genetics AG in Germany, a position I held since 2003. Before joining Icon Genetics in 1999 as Research Director of Halle Laboratory, I worked in several academic institutions including the Institute of Protein Research of Russian Academy of Sciences (1980-1982), Biological Research Centre of Hungarian Academy of Science (1989-1990) and Sainsbury Laboratory, John Innes Centre, Norwich, UK (1991-1999). I have over 20 years of research and management experience in the fields of plant molecular biology, plant genetics and biotechnology.

2. I have authored over 30 publications in the fields of plant biotechnology, molecular biology and plant genetics, the latest including: *Santi et al.*, (2006), "Protection

conferred by recombinant Yersinia pestis antigens produced by a rapid and scalable plant expression system", Proc. Natl. Acad. Sci. U.S.A, 103:861-866; *Marillonnet et al.*, (2005), "Systemic *Agrobacterium tumefaciens*-mediated transfection of viral replicons for efficient transient expression in plants", *Nat Biotechnol.*, 23(6):718-723; *Gils et al.*, (2005) "High-yield production of authentic human growth hormone using a plant virus-based expression system", *Plant Biotechnol. J.*, 3:613-620; *Gleba et al.*, (2005), "Magnifection--a new platform for expressing recombinant vaccines in plants", *Vaccine,* 23(17-18):2042-2048; *Klimyuk et al.*, (2005) "Production of recombinant proteins in Plants", in *"Modern Biopharmaceuticals"*, Ed. *Knaeblein*, Wiley-VCH, chapter 6, pp. 893-917; and *Marillonnet et al.*, (2004), "In planta engineering of viral RNA replicons: efficient assembly by recombination of DNA modules delivered by Agrobacterium", *Proc Natl Acad Sci U S A,* 101:6852-6857. In addition to my publications, I have also presented numerous lectures on the topic of plant biotechnology as invited speaker on international conferences, and am an inventor on more than 20 patents and patent applications.

4. As a named co-inventor of the present application, I have reviewed the United States Patent Office Communication mailed May 31, 2005, and I am familiar with the positions taken by the Examiner in this Office Action, particularly those based on "undue experimentation". I will address each of these in turn.

5. First, on pages 6-7 of the Office Action, the Examiner contends that Examples I-V give "rudimentary details", such that the claimed invention is simply not exemplified. This is not correct. Example I is very detailed and can be reproduced by a person in this industry. I personally designed the constructs described in the patent application and have overseen tests to reproduce Example I. These tests, performed by Icon scientists, resulted in finding an efficient transposition of non-autonomous transposon *dSpm* in *Orychophragmus* and *Brassica* to produce unstable hybrids obtained via cross-pollination. Similar experiments, not described in the application, were performed using protoplasts fusion between transgenic *Arabidopsis thaliana* (donor) and *Brassica juncea* (acceptor). We also performed experiments (not presented in the application) with two other plant species – *Nicotiana africana* (donor) and *Nicotiana tabacum* (acceptor), resulting in transposition in unstable progeny. While Examples II-V are of a prophetic nature, more recent experiments have been conducted under my general supervision, in which the protocols in these examples were followed, substantiating these results.

Briefly, we (Icon Genetics in collaboration with IPK, Gatersleben, Germany) investigated in detail the process of elimination of donor plant chromosomes from unstable hybrids formed by crosses between monocotyledonous wheat species and pearl millet. The results of this investigation were published in a highly reputable plant journal (Gernand *et al.*, 2005 "Uniparental chromosome elimination at mitosis and interphase of wheat and pearl millet crosses involves micronucleus formation, progressive heterochromatinization and DNA fragmentation", *Plant Cell*, 17:2431-2438). This demonstrates (and actually supports previously published data concerning unstable hybrids) that all pearl millet chromosomes were eliminated from unstable hybrid within 23 days after pollination. These results also demonstrate that: a) genomes of two parents co-exist in developing embryos for many cell divisions, thus for a long enough period of time for transposition or site-specific recombination to occur; and b) no traces of pearl millet chromatin were detected using a highly sensitive analysis in developing wheat embryo 23 days after cross-pollination, thus supporting previous observations. We also tested the efficiency of relatively new recombination systems based on phage C31 integrase in monocot species and showed that it is very efficient for site-specific recombination.

6. The Examiner contends there is a lack of guidance as to which parental plants to use. (Office Action p. 8-9.) However, there are many references throughout the specification specifying which pairs of plant species may be used to form unstable hybrids along with key references. For example, page 4 reads in part,

> In other preferred embodiments, the first plant, also referred to as to the donor or the clipboard species, is Tripsacum and in the second plant, also referred to as the recipient, is maize, wheat, barley or oat. In another preferred embodiment, the donor is Orychophragmus and the recipient is a crucifer such as canola. Other preferred donor/recipient pairs are: Glycine tomentella/ soybean, Solanum phreja/potato, maize/wheat, maize/barley, maize/oat, Pennisetum/wheat, Pennisetum/barley, Hordeum bulbosum/barley, Hordeum bulbosum/wheat, Nicotiana digluta/Nicotiana tabacum and Oryza minuta/rice.

Other examples are indicated in the text on page 8, lines 11-19:

> Insofar as dicotyledonous crops are concerned, the best studied case for potato is a hybridization between commercial varieties of potato, *Solanum tuberosum*, and a wild species *Solanum phureja*, that results in high frequency of haploid production as a result of early *phureja* chromosome elimination in hybrid embryo - Hougas, *et al.*, Crop. Sci. *4*:593-595 (1964); Clulow, *et al.*, *Theor. Appl. Genet. 82:*545-551 (1991). Regarding Canola/Rapeseed, somatic separation of the parental genomes in hybrids between *Brassica napus* and *Orychophragmus violaceous* is described in Li, Z., *et al.*, *Theor. Appl. Genet.* 91:131-136 (1995); Li, *et al.*, *Hereditas* 125: 69-75 (1996); Li, *et al.*, *Theor. Appl. Genet.* 96:251-265 (1998); Wu, J., *et al.*, *Plant Breeding 116*:251-257 (1997).

While these are not the only examples of plant pairs discussed in the application, based on these examples alone, it is clear that the application contains both examples of useful plant pairs for this invention, as well as indications as to which plant qualities and how to select for other plant pairs that are not disclosed in the application.

7. The Examiner has further contended on pages 9 and 11 of the Office Action that the specification lacks instruction regarding how to eliminate inoperable embodiments (crosses that do not achieve the intended result), and that "undue" random trial and error experimentation would be required to determine such inoperable embodiments. However, reduction of inoperable embodiments would not be a problem for those familiar with this art. It is well-known and standard procedure to make constructs providing the expression of a selection marker to indicate the desired recombination/transposition, resulting in an easy selection for such inoperable embodiments. Alternatively, simple and routine PCR-based screening can be performed to detect rescued embryos carrying the desired events, namely the nucleic acid of interest. Moreover, "undue" experimentation would not be necessary even to perform routine experiments because the information provided in the patent application reduces the nature and amount of screening, and teaches use of routine techniques to a volume necessary to practice any other technology in this art. The specification provides description and references for two key components of the invention, namely unstable hybrid formation, which in and of itself, is a well-established technology used by plant breeders to obtain haploid lines, and site-specific recombination and transposition, which techniques are also well-known in this industry as discussed above. Moreover, as one in this industry would know, based on the present specification, transposition can be used in the current invention for introducing sites for site-specific recombination, which would otherwise be difficult to transform by standard tissue

culture techniques using donor plant species. Such donor plants with recombination sites can be used to introduce any other sequence of interest via site-specific recombination through a second round of line conversion using an unstable hybrids approach. These standard techniques, which are all described in the specification, would provide enough guidance for a person in this art to perform these techniques without "undue" experimentation, as the Examiner asserts.

8. On pages 8 and 9-10, the Examiner contended that the specification lacks any examples of embodiments using recombinases/recombination sites, and in view of complexity of recombinases it would be unpredictable that any recombinase would meet the requirements of the present invention. Recombinases are routinely used in the field of plant biotechnology and do not present a limiting step in practicing this invention. The systems are, indeed, simple and work very well in higher eukaryotes including plants. Moreover, techniques using these kinds of recombinases and recombination sites are well-known. For example, Cre/loxP systems are taught in Sieburth *et al.*, *Development*, *125*:4303-4312 (1998). FLP/FRT systems are used in monocots, as exemplified in Lyznik *et al.*, *Nucleic Acids Res.*, *24*:3784-3789 (1996); Lyznik *et al.*, *Nucleic Acids Res.*, *21*:969-75 (1993); and Lyznik *et al.*, *Plant J.*, *8*:177-186 (1995). This same system is well-known for use in dicot plants as illustrated by Lloyd & Davies, *Mol Gen Genet.*, *242*:653-657 (1994). R/RS systems may also be used, as shown in Onouchi *et al.*, *Nucleic Acids Res.*, *19*:6373-6378 (1991); and Onouchi *et al.*, *Mol Gen Genet.*, *247*:653-660 (1995). While these are only some of the recombinase/recombinase systems that may be used, they illustrate that recombinases and recombinase systems are well-known and used in this industry.

9. On page 10 of the Office Action, the Examiner asserted that there is no indication as to the proper time, place, concentration and proper phase of cell cycle to use with the recombinase, in order to produce a haploid transgenic plant. As one in this art would appreciate however, these limitations are dependent upon the recombinase used. Thus, the choice of a particular recominase/recombinase system described in the specification, or any other well-known system, would determine such limitations, which would be well-known to a person in this industry.

10. Finally, the Examiner contended that there is a lack of instruction as to which regulatory sequences to use for expression of recombination partners in this invention.

(Office Action p. 11-12). Regulatory sequences are not a restricting feature of this invention, as "the "active" phase of the recombinase of choice is already restricted spatially and temporally to the unstable hybrid state". In other words, the system is active when two recombinant components of it inserted in two different genomes (genome of donor plant and genome of acceptor plant) are brought together in an unstable hybrid. The most unusual case, when two components (transposase source and non-autonomous transposon with recombination site and/or with selectable marker gene are inserted into the genome of donor plant, has been exemplified in the specification. In the examples, the constitutive promoters (p35S; pSpm) were used to drive the expression of transposase source. This is to show how the sequence of interest (*e.g.*, acceptor site) is introduced into difficult-to-transform (or non-transformable) acceptor plant species. Once a non-autonomous transposone is integrated into the genome of an acceptor plant, it will be stabilized as the source of transposase will be segregated away in unstable hybrid cells together with the genome of donor plant. Otherwise, those familiar with the art would have understood that the donor plant can provide, for example, the gene of interest flanked by recombination sites recognized by site-specific recombinase. An acceptor plant can provide recombinase under control of a constitutive promoter, where the recombinase gene is separated from constitutive promoter by yet another recombination site (acceptor site). The acceptor site is usually too short to interfere with expression of the recombinase, but integration of the gene of interest from donor plant via site-specific recombination within the site will turn off the expression of the recombinase, thus stabilizing the plant recombination event.

11. In addition to the foregoing, based upon my experiences in the field of plant biotechnology, which includes more than 20 years, it is my belief that our invention that is claimed would be practiced by one skilled in the art using the present specification and examples without "undue experimentation". While some routine experimentation might be used such as for screening purposes to determine which parental plants would be most conducive to the intended outcome, or a screening to determine which progeny have the desired transgene, but these are well-known techniques that any person in this art would expect to perform in conducting plant transformation.

12. I declare under penalty of perjury that the foregoing is true and correct.

Dated: January 31st, 2006

V. Klimyuk

VICTOR KLIMYUK, Ph.D.

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